

CLAIMS

1. A variant of a parent Termamyl-like α -amylase, which variant α -amylase has been altered in comparison to the parent α -amylase in one or more solvent exposed amino acid residues on the surface of the α -amylase to increase the overall hydrophobicity of the α -amylase and/or to increase the overall numbers of methyl groups in the sidechains of said solvent exposed amino acid residues on the surface.

10 2. The variant according to claim 1, wherein one or more solvent exposed amino acid residues on a concav surface with inwards bend are altered to more hydrophobic amino acid residues.

15 3. The variant according to claim 1, wherein one or more solvent exposed amino acid residues on a convex surface are altered to increase the number of methyl groups in the sidechain.

20 4. A variant of a parent Termamyl-like α -amylase, comprising an alteration at one or more positions selected from the group of: E376, S417, A420, S356, Y358; wherein (a) the alteration(s) are independently (i) an insertion of an amino acid downstream of the amino acid which occupies the position, (ii) a deletion of the amino acid which occupies the position, or (iii) a substitution of the amino acid which occupies the position with a different amino acid, (b) the variant has α -amylase activity and (c) each position corresponds to a position of the amino acid sequence of the parent Termamyl-like α -amylase having the amino acid sequence of SEQ ID NO: 4.

25 5. The variant according to claim 4, which variant has an alteration in one or more solvent exposed amino acid residues as defined in any of claims 1-3.

1 According to Claim 1

6. The variant of any of claims 1-5, wherein the parent Termamyl-like α -amylase is derived from a strain of *B. licheniformis*, *B. amyloliquefaciens*, *B. stearothermophilus*, *Bacillus* sp. NCIB 12289, NCIB 12512, NCIB 12513 or DSM 9375.

2 According

7. The variant according to claim 6, wherein the parent α -amylase is derived from *B. licheniformis* strain ATCC 27811.

10 8. The variant according to claims 1-6, wherein the parent Termamyl-like α -amylase is any of the α -amylases selected from the group depicted in SEQ ID NOS: 1, 2, 3, 4, 5, 6, 7, and 8.

15 9. The variant according to any of claims 1-8, wherein the parent Termamyl-like α -amylase has an amino acid sequence which has a degree of identity to SEQ ID NO: 4 of at least 65%, preferably 70%, more preferably at least 80%, even more preferably at least about 90%, even more preferably at least 95%, even more preferably at least 97%, and even more preferably at least 97%.

20 10. The variant according to any of claims 1-9, wherein the parent Termamyl-like α -amylase is encoded by a nucleic acid sequence which hybridizes under medium, preferred high stringency conditions, with the nucleic acid sequence of SEQ ID NO: 12.

25 11. The variant according to claims 1-10, wherein the parent Termamyl-like α -amylase is a hybrid of the *B. licheniformis* α -amylase shown in SEQ ID NO: 4 and *B. amyloliquefaciens* α -amylase shown in SEQ ID NO: 5.

12. The variant according to claim 11, wherein the parent hybrid Termayl-like α -amylase is LE174.

13. The variant according to any of claims 1-12, wherein the parent α -amylase further has a mutation in one or more of the following positions: K176, I201 and H205 (using the numbering in SEQ ID NO: 4).

5

14. The variant according to claim 13, wherein the parent α -amylase has one or more the following substitutions: K176R, I201F and/or H205N (using the numbering in SEQ ID NO: 4).

10 15. The variant according to claim 14, wherein the parent α -amylase has the following substitutions: K176R+I201F+H205N (using the numbering in SEQ ID NO: 4).

15 16. The variant according to claims 1 to 15, wherein the variant has increased stability at pHs below 7.0 (acidic pH) and/or at low calcium concentration and/or at temperatures in the range from 95 to 160°C (high temperatures) relative to the parent α -amylase.

20 17. The variant according to any of claims 1 to 16, which variant has one or more of the following substitutions: E376K, S417T, A420Q, R, S356A, Y358F.

A 18. A DNA construct comprising a DNA sequence encoding an α -amylase variant according to any one of claims 1 to 17.

19. A recombinant expression vector which carries a DNA construct according to claim 18.

20 20. A cell which is transformed with a DNA construct according to claim 18 or a vector according to claim 19.

21. A cell according to claim 20, which is a microorganism.

22. A cell according to claim 21, which is a bacterium or a fungus.

23. The cell according to claim 22, which is a grampositive bacterium such as Bacillus subtilis, Bacillus licheniformis, Bacillus latus, Bacillus brevis, Bacillus stearothermophilus,
s Bacillus alkalophilus, Bacillus amyloliquefaciens, Bacillus coagulans, Bacillus circulans, Bacillus laetus or Bacillus thuringiensis.

24. A detergent additive comprising an α -amylase variant according to any one of claims 1 to 17, optionally in the form of a non-dusting granulate, stabilised liquid or protected enzyme.

25. A detergent additive according to claim 24 which contains 0.02-200 mg of enzyme protein/g of the additive.

15 26. A detergent additive according to claims 24 or 25, which additionally comprises another enzyme such as a protease, a lipase, a peroxidase, another amylolytic enzyme and/or a cellulase.

20 27. A detergent composition comprising an α -amylase variant according to any of claims 1 to 17.

28. A detergent composition according to claim 27 which additionally comprises another enzyme such as a protease, a lipase, a peroxidase, another amylolytic enzyme and/or a cellulase.

20 29. A manual or automatic dishwashing detergent composition comprising an α -amylase variant according to any of claims 1 to 17.

30 30. A dishwashing detergent composition according to claim 29 which additionally comprises another enzyme such as a protease, a lipase, a peroxidase, another amylolytic enzyme and/or a cellulase.

31. A manual or automatic laundry washing composition comprising

an α -amylase variant according to any of claims 1 to 17.

32. A laundry washing composition according to claim 31, which additionally comprises another enzyme such as a protease, a 5 lipase, a peroxidase, an amylolytic enzyme and/or a cellulase.

33. A composition comprising:

(i) a mixture of the α -amylase from *B. licheniformis* having the sequence shown in SEQ ID NO: 4 with one or more variants 10 according to any of claims 1 to 17 derived from (as the parent Termamyl-like α -amylase) the *B. stearothermophilus* α -amylase having the sequence shown in SEQ ID NO: 3; or

(ii) a mixture of the α -amylase from *B. stearothermophilus* having the sequence shown in SEQ ID NO: 3 with one or more variants 15 according to any of claims 1 to 17 derived from one or more other parent Termamyl-like α -amylases; or

(iii) a mixture of one or more variants according any of claims 20 1 to 17 derived from (as the parent Termamyl-like α -amylase) the *B. stearothermophilus* α -amylase having the sequence shown in SEQ ID NO: 3 with one or more variants according to the invention derived from one or more other parent Termamyl-like α -amylases.

34. The composition comprising a variant of any of claims 1 to 17 wherein the parent α -amylase is a hybrid alpha-amylase 25 comprising a N-terminal part of the *B. amyloliquefaciens* α -amylase shown in SEQ ID NO: 5 and a C-terminal part of the *B. licheniformis* α -amylase shown in SEQ ID NO: 4.

35. The composition according to claim 34, wherein the parent 30 hybrid Termamyl-like α -amylase is LE174

36. The composition according to claims 35, wherein the parent Termamyl-like α -amylase is LE174 with an alteration in one or more of the following positions: K176, I201 and H205.

37. The composition according to claims 36, wherein the parent Termamyl-like α -amylase is LE174 with one or more of the following substitutions: K176R, I201F and H205N.

5

38. Use of an α -amylase variant according to any one of claims 1 to 17 or a composition according to claims 33 to 37 for washing and/or dishwashing.

10 39. Use of an α -amylase variant according to any one of claims 1 to 17 or a composition according to claims 33 to 37 for textile desizing.

DRAFTING
0 10 20 30 40 50 60 70 80 90 100

40. Use of an α -amylase variant according to any of claims 1 to 17 or a composition according to claims 33 to 37 for starch liquefaction.

41. A method for generating a variant of a parent Termamyl-like α -amylase, which variant exhibits increased stability at high 20 temperatures relative to the parent, the method comprising:

(a) subjecting a DNA sequence encoding the parent Termamyl-like α -amylase to random mutagenesis,

(b) expressing the mutated DNA sequence obtained in step (a) in a host cell, and

25 (c) screening for host cells expressing a mutated α -amylase which has increased stability at high temperatures relative to the parent Termamyl-like α -amylase.

add
b4
b5